Pathogenesis and prevention of cardiovascular disease in patients with chronic kidney disease

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Patients with chronic kidney disease (CKD) have an unacceptably high risk of premature death, primarily as a result of cardiovascular disease (CVD) [1]. Several large, randomised trials in end stage renal disease (ESRD) patients have consistently shown no survival benefit from multiple new treatment strategies aimed at reducing CV disease. Earlier stages of CKD are common and are also associated with an increased risk of developing CVD. Traditional cardiovascular risk factors such as, age, dyslipidaemia, hypertension, diabetes mellitus, smoking and sedentary lifestyle, do not fully explain the cardiovascular mortality in these patients. The so-called novel risk factors such as increased oxidative stress, asymmetric dimethylarginine (ADMA), plasma adipokines, plasma homocysteine and DNA-hypomethylation are prevalent and thought to play an important role in development of atherosclerosis in CKD patients. However, the exact role of these factors in the pathogenesis of CV disease in CKD patients has not yet been elucidated. In addition, only a few cardiovascular intervention studies have been done in patients with mild to moderate CKD, whereas most of the large intervention trials with statins have excluded patients with moderate renal failure. Therefore, many important questions remained unanswered in this unfortunate patient group.

Anti-oxidant Therapy In chronic renal insufficiency (ATIC) study

With the aim of answering at least some of the above mentioned questions, in 2001 we started the Anti-oxidant Therapy In Chronic renal insufficiency (ATIC) study, a randomised, double-blind, placebo-controlled trial. We randomised 93 non-diabetic patients with mild to moderate chronic kidney disease (creatinine clearance 15-70 ml/min per 1.73 m^2 determined by Cockcroft-Gault formula), who were free of manifest atherosclerotic arterial disease, to receive a regimen that included pravastatin 40mg/day followed by α tocopherol acetate (vitamin E) 300mg/day and homocystiene lowering therapy (folic acid 5mg/day, pyridoxine hydrochloride 100mg/day, cyanocobalamin 1 mg/day) or matching placebo tablets for each consecutive six months period. The primary aim of the study was to evaluate the effect of this stepwise treatment strategy on common carotid artery intima-media thickness (CCA-IMT) and brachial artery flow-mediated vasodilatation (BA-FMD), both strong surrogate markers of cardiovascular disease. We further decided to evaluate the effects of this strategy on albuminuria and estimated glomerular filtration rate (eGFR). We also planned to use the baseline data of this study to evaluate the associations between renal function and several non-traditional risk factors such as asymmetric dimethylarginine (ADMA), plasma adipokines (plasma adiponectin and plasma leptin), and global leukocyte DNA hypomethylation. We also

intended to evaluate the associations between these novel risk factors and CCA-IMT and thereby to get some insight into the possible pathophysiological role these factors play in excess cardiovascular risk in CKD patients. In addition, we decided to assess the effect of this treatment strategy on plasma ADMA levels and global leukocyte DNA-methylation and to study whether eventual reduction in these risk factors/markers could explain the beneficial effects (if any) of this therapeutic strategy on the measured surrogate endpoints.

Why oxidative stress lowering and what was the rationale behind this peculiar design?

During the design of the study we postulated, on the basis of a few published studies, that the high cardiovascular mortality rates in ESRD patients could be partly explained by enhanced oxidative stress [2-4] and that the oxidative stress hypothesis should be considered as a unifying concept of increased cardiovascular disease in CKD patients [5]. It was already known that increased oxidative stress occurs in early stages of CKD [6] and oxidation of low-density lipoprotein (LDL) is thought to be a key step in the initiation of the early atherosclerotic lesion [7]. Therefore, we decided to look for interventions which would create oxidative stress reduction in our population.

In 1996, Stephens *et al* published the results of Cambridge Heart Antioxidant (CHAOS) study in which a population of patients with angiographically proven coronary atherosclerosis was treated with alpha-tocopherol 800 IU in a randomised controlled manner. The treatment significantly reduced the risk of developing the primary endpoint of cardiovascular death and non-fatal myocardial infarction [8]. In addition, although most of the interventions aimed at reducing cardiovascular disease in ESRD patients yielded disappointing results, the SPACE-study, in which 196 patients with ESRD who were treated with 800 IU vitamin E in a randomised controlled fashion, demonstrated encouraging results on composite cardiovascular endpoints and myocardial infarction [9]. It was also shown that plasma homocysteine was strongly related to the renal function and that virtually all ESRD patients had elevated homocysteine levels which also predicted cardiovascular outcomes in these patients [10]. It was further postulated that the vasculotoxic effects of homocysteine were caused by increased oxidative stress [11]. In addition, studies in various populations demonstrated that statins decreased cardiovascular endpoints spectacularly [12;13].

After considering all these facts thoroughly, we decided to use a strategy which consisted of pravastatin which was thought to reduce the substrate LDL-cholesterol and thereby reduce oxidized LDL, vitamin E which acts as a free-radical scavenger to reduce free oxygen radicals and a combination of B-vitamins to reduce homocysteine, because high homocysteine was

thought to increase oxidative stress. We decided to use these treatment modalities on top of each other on the one hand to reduce the number of people needed to perform the study and on the other hand to achieve a maximum oxidative stress reduction (benefit) in the treatment arm and thereby increase the power of the study. Because we thought that the maximum effect of each treatment would be achieved in six months, we decided to add each new intervention every six months. In addition, even at that time it was known that there was an unequivocal relationship between hypertension and progression of CKD, and that inhibition of the renin-angiotensin system with ACE-inhibitors would lead to a significant reduction in decline in eGFR and cardiovascular complications [14]. Therefore, we decided to control the blood pressure in both the treatment and control arm aggressively (<140/90mmHg) and all the subjects who were not on ACE-inhibitors or ARBs were put on ACE-inhibitors at inclusion. Although currently lower blood pressure targets are recommended, especially in patients with proteinuria > 1g/day, during the design of the study many guidelines recommended 140/90 mmHg as the target blood pressure, and we decided to adhere to this target strictly during the study period.

What have we learned from the ATIC study?

In chapter 3 of this thesis we demonstrate, for the first time, that plasma adiponectin had a significant, non-linear, inverse association with eGFR and that in the multivariate analysis eGFR had the strongest correlation with plasma adiponectin. In addition, adiponectin had no influence on the significant association between estimated glomerular filtration rate (eGFR) and a surrogate marker of endothelial dysfunction (plasma von Willebrand factor) and a marker of leukocyte-endothelial cell adhesion [soluble vascular leukocyte cell adhesion molecule-1 (sVCAM-1)]. We, therefore, postulated that the increased adiponectin levels in CKD patients are primarily a reflection of impaired kidney function. Although renal function strongly predicted plasma leptin in our population, body mass index and insulin resistance also had a strong association with plasma leptin in univariate and multivariate analysis. Plasma leptin also did not explain the known associations between kidney function and endothelial dysfunction and leukocyte-endothelial cell adhesion. Future studies are needed to clarify the role of leptin in CKD patients.

Adipose tissue has a physiological role beyond mere storage of fat and recent interest has focused on the role of adipokines, such as adiponectin and leptin, both as protectors and promoters of vascular disease in CKD [15;16]. However, the pathophysiological role of

adiponectin in excess CVD in CKD patients is not clear, as published studies have shown contradicting results [17]. For example, while Guebre-Egziabher *et al.* reported that the increase in adiponectin in patients with CKD is explained primarily by patient's body composition and the altered metabolic parameters[18], Mitsnefes *et al.* attributed this increase primarily to the decline in kidney function[19]. While one study reported that lower adiponectin levels are associated with increased risk of cardiovascular events in CKD patients, [20] others reported that high rather than low adiponectin levels predicts mortality in both CKD and congestive heart failure[21]. In addition, the functional role of increased leptin in CKD patients is also unclear[22]. We planned to investigate the association between renal function and plasma leptin and adiponectin in a population with a wide range of eGFR so that we could shed some light on these matters. Therefore, we pooled the baseline data from two studies which were being performed in the same centres as the ATIC study. Our data show that eGFR was the main determinant of plasma adiponectin and also that plasma adiponectin and leptin did not explain the known associations between eGFR and endothelial dysfunction. The limitations of the study design are described in detail in chapter 3.

In chapter 4, using baseline data from the ATIC-study, we demonstrated that, in patients with mild to moderate renal failure, eGFR was inversely associated with plasma ADMA level. We also demonstrated, for the first time, that plasma ADMA was in turn associated with CCA-IMT and plasma soluble vascular cell adhesion molecule-1. We conclude that plasma ADMA may be one of the mechanisms that link mild to moderate renal failure with cardiovascular disease. During the design of the ATIC study it was already known that plasma ADMA concentrations were high and were strongly and independently related to CCA-IMT in patients with ESRD [23]. However, data on the association between plasma ADMA and CCA-IMT were not available at that moment, and there were conflicting data on the association between eGFR and plasma ADMA in patients with mild to moderate kidney failure[24;25]. Thus our findings were novel and thought provoking.

In chapter 5 we demonstrate that in patients with mild to moderate CKD, the global DNA-methylation was not associated with renal function or with CCA-IMT or BA-FMD. In addition we also showed that the above-mentioned treatment strategy had no influence on DNA- methylation in this population. Therefore, we concluded that DNA-hypomethylation probably has no significant role in the pathogenesis of cardiovascular disease in patients with CKD. During the design of the study, hyperhomocysteinaemia was strongly implicated in the development and progression of atherothrombotic vascular disease [26]. The pathophysiological

explanation for this link was initially thought to be a direct vasculotoxic effect of homocysteine itself [27]. However, an alternative view was that hyperhomocysteinaemia itself is not harmful but indirectly inhibits methyl fluxes during transmethylation of methionine and thereby leads to a decreased methylation of DNA. Global DNA-hypomethylation was thought to be associated with various diseases including atherosclerotic vascular disease[28]. Global DNA-hypomethylation has been demonstrated in ESRD patients [29] and was implicated as an important candidate contributing to CVD in these patients [30]. However, it was thought that leukocyte DNA-hypomethylation in ESRD patients may be caused by leukocyte activation on dialysis membranes and data on patients with mild to moderate renal failure and DNA-hypomethylation were inconclusive [31]. As mentioned earlier, results of many studies to examine the effects of therapeutic homocysteine lowering has been disappointing. We, unfortunately, also could not demonstrate an association between DNA-hypomethylation and eGFR and our treatment strategy also did not alter global DNA-methylation. Our results suggest that the role of global DNA-hypomethylation as a risk factor for CVD in patients with CKD, if any, is limited.

In chapter 6 we demonstrated that 18 months of the stepwise treatment strategy, on top of well-controlled blood pressure, achieved a significant reduction in CCA-IMT and significant increase in BA-FMD in the active treatment arm after adjustments for baseline values. Increased urinary albumin excretion was also attenuated by the treatment strategy although there were no observed beneficial effects on renal function (eGFR). Unfortunately, the individual effects of each intervention could not be determined and also the study was not powered to detect a clinically important difference in hard cardiovascular endpoints. We concluded that in patients with mild to moderate chronic kidney disease, 18 months of an oxidative stress lowering treatment strategy along with well-controlled blood pressure reduced CCA-IMT and urinary albumin excretion and increased BA-FMD. In addition, the treatment strategy, especially pravastatin, was associated with a low rate of adverse events.

In chapter 7 we demonstrate that the above-mentioned treatment strategy had no significant effect on plasma ADMA levels. However, analysis of separate treatment effects suggested that vitamin E significantly lowered ADMA in the treatment group compared to the placebo group. This effect disappeared after addition of homocysteine lowering treatment to the treatment arm. We concluded that our treatment strategy had no effect on plasma ADMA levels, and hence the observed reduction in CCA-IMT and improvement BA-FMD in our

population could not be explained by a change in ADMA levels. In the next paragraph, we describe in detail the implications of our findings.

What were the drawbacks in the ATIC study?

The most accurate way to investigate the determinants of the excess cardiovascular risk in patients with CKD, compared to patients without CKD, is to perform a prospective population-based cohort study with exclusion of patients with cardiovascular disease at the baseline. Although the ATIC study was a prospective study we designed this study primarily to investigate the treatment effects. Although we have published three papers describing cross sectional associations using baseline data of our study, these findings do not permit any final conclusions with regard to the causality of the described associations. In addition, we decided to include patients using ACE-inhibitors or angiotensin receptor blockers (ARB) and patients not using these agents were put on ACE-inhibitors before the initiation of the study. Oxidative stress can also be reduced by ACE-inhibitors and ARBs [32]. ACE-inhibitors also lower plasma ADMA [33;34]. This may have influenced all our cross sectional analyses and may also have reduced the power of our study. However, since the use of ACE-inhibitors in CKD, especially in the presence of any level of proteinuria, was considered standard treatment even during the design of our study, it made sense to evaluate our anti-oxidant strategy on top of treatment with ACE-inhibitors. Although diabetes mellitus is expected to be one of the major causes of CKD in the future we excluded patients with diabetes mellitus. By excluding patients with diabetes as well as patients with previous CVD, we expected to recruit a population with increased oxidative stress primarily caused by their kidney failure. We, therefore, studied a selected population of patients with mild-to-moderate CKD and this study unfortunately had limited power and was of too short duration to detect an effect on clinical cardiovascular endpoints.

In the last few years results of large, randomised, controlled trials with vitamin E and homocysteine lowering have not shown any beneficial effects on cardiovascular events in different populations [35-38]. However, during the period we designed our study the available information at that time suggested, these vitamins to have beneficial effects in patients with renal failure because these patients were known to have increased oxidative stress and small studies with vitamin E in dialysis patients at that time showed some promising results [39]. At the same time, many studies showed an undeniable link between homocysteine , renal function and cardiovascular disease, and during that time homocysteine lowering was thought to be one of the best potential interventions to reduce CV disease in CKD patients [10;39]. We

decided to use the treatment strategies on top of each other with six monthly intervals and planned to evaluate the effects of individual treatments separately. We expected (in retrospect, wrongly) the maximum effect of each intervention to be achieved within six months after the given intervention, and/or that the additional effect of the next step would be clearly distinguishable from the effects of the previous step. Lowering of CCA-IMT and improvement in BA-FMD were observed during the whole study period (chapter 6). In retrospect, we were unable to draw any conclusions on individual effects of these interventions. The treatment modalities of the present study certainly have effects independent of oxidative stress lowering. In addition, there is no gold standard for the detection of oxidative stress in vivo. We measured the oxidized LDL concentration which is unfortunately also influenced by the basal LDL-cholesterol levels. We measured total malondialdehyde (MDA) levels in plasma, i.e. the most studied product of polyunsaturated fatty acid peroxidation. However, the specificity of commonly used tests to determine MDA is low as these tests also measure several compounds other than MDA[40]. F2-isoprostanes have recently gained recognition as reliable markers of oxidative stress[41]. At the moment we are in the process of introducing the measurement of F2-isoprostanes in our laboratory and we hope to measure the F2-isoprostanes in our study population in the near future**.** Until then, we cannot draw any conclusions whether the observed improvements were the results of oxidative stress lowering or other effects such as reduction of lipid levels.

Where should we go from here?

While it is widely known that patients with ESRD are at greatly increased risk of developing CV disease, it is not well known among the general physicians, that patients with only mild to moderate kidney disease are also at increased risk. The clear association between slightly reduced kidney function and cardiovascular risk may, at least partly, be the result of a relationship between total atherosclerotic burden and decreased renal function, because intrarenal atherosclerosis (ischaemic renal disease) is a common cause of reduced renal function in patients with atherosclerosis. However, there are clear indications that impaired eGFR is an independent risk factor for developing cardiovascular disease in the general population [42]. In spite of this, not a single risk calculator, which are recommended for physicians, uses eGFR to calculate the risk of developing CV disease. This is puzzling. In our opinion, most patients with mild kidney disease are not diagnosed and most physicians do not measure creatinine clearance during routine check ups. Measurement of only plasma creatinine can underestimate the renal function especially in old and thin patients. Thus mild

to moderate kidney failure is, in our opinion, underdiagnosed. Several guidelines have been formulated, both nationally and internationally, to assist the physicians to diagnose and treat patients with kidney disease. However it is well known that patients do not reach treatment goals formulated in these guidelines [43]. This also applies to patients with kidney disease [44;45]. Thus, apart from being underdiagnosed, CKD is also not treated properly. Furthermore, most of these patients come to nephrologists when they reach stage 4 which is in my opinion already too late for cardiovascular preventive measures. In patients with diabetes mellitus and heart failure a multifactorial intervention significantly improved metabolic control and reduced cardiovascular events significantly[46;47] Therefore, much benefit may be achieved effectively with a multifactorial approach addressing risk factors such as blood pressure, serum lipids, dietary meausres, physical exercise et cetera in mild to moderate CKD. The MASTERPLAN study, a randomised, controlled trial examining the effect of such a multifactorial approach is already underway in The Netherlands and hopefully this study will shed more light on this matter in the future. In the mean time, general physicians and practitioners should be urged to consider mild to moderate CKD a cardiovascular risk factor and perhaps routine creatinine measurement should automatically generate estimated GFR which would make it easy for physicians to diagnose CKD.

The ATIC study is one of the first clinical trials primarily designed to examine the effects of a treatment strategy which included pravastatin on surrogate markers of CV disease in CKD patients. We did demonstrate strong favourable effects on these markers with our treatment strategy and, in my opinion pravastatin certainly played a key role in these effects. Also very few subjects stopped the therapy because of side-effects. However, long term studies with clinical endpoints are needed to confirm these results and some are already underway. One of the largest is the SHARP study, a randomised, double blind, placebo-controlled trial which investigates the effects of cholesterol reduction with simvastatin and ezetimibe in around 6000 patients with moderate CKD (plasma creatinine > 150 μmol/L in men and 130 μmol/L in women) and 3000 patients with ESRD on major vascular events; the estimated study completion date is July 2010 (ClinicalTrials.gov Identifier: NCT00125593). In addition, the Heart Outcomes Prevention Evaluation-3 (HOPE-3 study), which examines the effects of combined blood pressure lowering and lipid lowering with rosuvastatin 10mg a day, is underway at this moment, and this study will also include patients with CKD (ClinicalTrials.gov Identifier: NCT00468923). Unfortunately studies with homocysteine lowering in various populations including CKD patients have given disappointing results [48].

The SPACE study, which assessed the effect of vitamin E 800 iu/day versus placebo in 196 haemodialysis patients with preexisting CV disease over a median period of 519 days, demonstrated a significant reduction in cardiovascular endpoints. However, the Heart Outcomes Prevention Evaluation study (HOPE study), which included 993 subjects with serum creatinine concentration between 125 and 200 µmol/L, found no effect on cardiovascular outcomes of treatment with vitamin E 400 iu/day compared to placebo. The apparent disparity in findings between SPACE and HOPE may be explained by the higher dose of vitamin E used, the greater severity of kidney disease and the three fold higher CV event rate in the SPACE trial. Many guideline groups have therefore considered the SPACE trial preliminary and demanded further studies before recommending vitamin E therapy routinely for CKD patients. A large study with antioxidant combination vitamin E and alpha lipoic acid in stage 3 and 4 CKD patients is underway (ClinicalTrials.gov Identifier: NCT00308971).

There is growing evidence to support the hypothesis that high plasma ADMA, an endogenous inhibitor of NO synthase, is associated with increased risk of CVD [49]. Although we could demonstrate a significant reduction in CCA-IMT and a significant increase in BA-FMD our treatment strategy had no effect on plasma ADMA. Therefore, reduction in ADMA could not explain these favourable effects. This is on the one hand disappointing and on the other hand encouraging. The question remains whether pharmacological reduction in ADMA would lead to more favourable effects on these vascular parameters. Small studies have demonstrated that drugs such as fenofibrate[50;51], thiazolidinediones[52], ACE-inhibitors and ARBs[34;53], vitamin E and even rosuvastatin[54]can reduce ADMA[55]. We did demonstrate a significant reduction in ADMA during treatment with vitamin E which disappeared after addition of homocysteine lowering therapy. However, large scale studies to examine the effects of ADMA reduction on CV events are, to our knowledge, not underway. In our opinion, long term studies with vitamin E or other agents to examine the effect of possible ADMA reduction on vascular endpoints are of great interest.

Recent studies show that the adipose tissue is a complex organ with functions far beyond the storage of fat, and secretes adipokines such as adiponectin and leptin[56]. Evidence suggests that these signaling molecules are linked to insulin resistance, systemic inflammation and uraemic anorexia in patients with CKD[57;58]. As discussed in detail in chapter 3, the exact pathophysiological role of these adipokines in CKD is far from clear. Further research is needed to investigate the complex interactions between adipokines signaling networks and their effects on vascular health and outcome in CKD.

In addition, there are exciting new fields such as the role of fibroblast growth factor 23 (FGF 23) and vitamin D deficiency in chronic kidney disease[59]. Several factors including parathyroid hormone (PTH) and vitamin D play a critical role in maintaining plasma phosphate levels [60]. FGF 23 has recently been identified as an important regulator of systemic phosphate balance[61]. Under physiological conditions FGF23 promotes phosphaturia and suppresses the 1 ά-hydroxylase activity, thus leading to a reduction in 1,25-dihydroxyvitamin D levels. The phosphate balance is altered in CKD and in these patients very high levels of FGF23 have been demonstrated[62]. As the number of viable nephrons decreases in CKD, in spite of the high FGF23 the net phosphate excretion does not increase sufficiently. This high phosphate level in combination with the reduction in 1,25-dihydroxyvitamin D levels leads to secondary hyperparathyroidism. A strong association has been described between hyperphosphataemia, hyperparathyroidism and CV disease in CKD[63]. This increased CV disease is probably caused by increased vascular calcification and evidence is emerging that optimizing treatment of calcium and phosphate alterations may decrease CV risk in CKD patients[64]. However, the exact role of these new factors such as FGF23 in patients with CKD has not yet been elucidated and is in our opinion an exciting challenge for the future.

The terms risk factor reversal, paradoxical risk factors or reverse epidemiology refer to alterations in the normal relation between risk factors and clinical outcomes. In particular populations this abnormal relation can be so severe that this can result in more or less the exact opposite or reversal of the usual association between a risk factor and clinical outcome that is found in the general population. Such risk factor reversal is commonly observed in patients with advanced CKD[65]. This phenomenon of reverse epidemiology makes it sometimes difficult to target traditional risk factors in an effective manner because determination of an optimal target for risk factors such as blood pressure and LDL-cholesterol has become uncertain, especially in patients with advanced stages of CKD. Therefore, randomised controlled trials will certainly be necessary to ascertain the optimal levels for these risk factors in CKD and ESRD patients.

In spite of a long and hard journey by many research groups the puzzle of increased cardiovascular disease in CKD has certainly not yet been resolved. The ATIC study has left us with more questions than answers. The journey is, in our opinion, far from over.

Reference List

1. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998; 32: S112-S119

2. Loughrey CM, Young IS, Lightbody JH, McMaster D, McNamee PT, Trimble ER. Oxidative stress in haemodialysis. *QJM* 1994; 87: 679-683

3. Toborek M, Wasik T, Drozdz M, Klin M, Magner-Wrobel K, Kopieczna-Grzebieniak E. Effect of hemodialysis on lipid peroxidation and antioxidant system in patients with chronic renal failure. *Metabolism* 1992; 41: 1229-1232

4. Zima T, Haragsim L, Stipek S, Bartova V, Nemecek K. Lipid peroxidation on dialysis membranes. *Biochem Mol Biol Int* 1993; 29: 531-537

5. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002; 62: 1524-1538

6. Oberg BP, McMenamin E, Lucas FL *et al*. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 2004; 65: 1009-1016

7. Parthasarathy S, Santanam N, Auge N. Oxidized low-density lipoprotein, a two-faced Janus in coronary artery disease? *Biochem Pharmacol* 1998; 56: 279-284

8. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; 347: 781-786

9. Boaz M, Smetana S, Weinstein T *et al*. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 2000; 356: 1213-1218

10. Mallamaci F, Zoccali C, Tripepi G *et al*. Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients. *Kidney Int* 2002; 61: 609-614

11. Kanani PM, Sinkey CA, Browning RL, Allaman M, Knapp HR, Haynes WG. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocyst(e)inemia in humans. *Circulation* 1999; 100: 1161-1168

12. Shepherd J, Cobbe SM, Ford I *et al*. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995; 333: 1301-1307

13. Sacks FM, Pfeffer MA, Moye LA *et al*. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996; 335: 1001-1009

14. Brenner BM. Retarding the progression of renal disease. *Kidney Int* 2003; 64: 370-378

15. Zoccali C, Mallamaci F, Tripepi G *et al*. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 2002; 13: 134-141

16. Scholze A, Rattensperger D, Zidek W, Tepel M. Low serum leptin predicts mortality in patients with chronic kidney disease stage 5. *Obesity (Silver Spring)* 2007; 15: 1617-1622

17. Mitsnefes M, Kartal J, Khoury P, Daniels S. Adiponectin in children with chronic kidney disease: role of adiposity and kidney dysfunction. *Clin J Am Soc Nephrol* 2007; 2: 46-50

18. Guebre-Egziabher F, Bernhard J, Funahashi T, Hadj-Aissa A, Fouque D. Adiponectin in chronic kidney disease is related more to metabolic disturbances than to decline in renal function. *Nephrol Dial Transplant* 2005; 20: 129-134

19. Mitsnefes M, Kartal J, Khoury P, Daniels S. Adiponectin in children with chronic kidney disease: role of adiposity and kidney dysfunction. *Clin J Am Soc Nephrol* 2007; 2: 46-50

20. Zoccali C, Mallamaci F, Tripepi G *et al*. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 2002; 13: 134-141

21. Menon V, Li L, Wang X *et al*. Adiponectin and mortality in patients with chronic kidney disease. *J Am Soc Nephrol* 2006; 17: 2599-2606

22. Axelsson J, Stenvinkel P. Role of fat mass and adipokines in chronic kidney disease. *Curr Opin Nephrol Hypertens* 2008; 17: 25-31

23. Zoccali C, Benedetto FA, Maas R *et al*. Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. *J Am Soc Nephrol* 2002; 13: 490-496

24. Wahbi N, Dalton RN, Turner C, Denton M, Abbs I, Swaminathan R. Dimethylarginines in chronic renal failure. *J Clin Pathol* 2001; 54: 470-473

25. Kielstein JT, Boger RH, Bode-Boger SM *et al*. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. *J Am Soc Nephrol* 2002; 13: 170-176

26. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002; 288: 2015-2022

27. van Guldener C, Stehouwer CD. Hyperhomocysteinaemia and vascular disease--a role for DNA hypomethylation? *Lancet* 2003; 361: 1668-1669

28. Wilson AS, Power BE, Molloy PL. DNA hypomethylation and human diseases. *Biochim Biophys Acta* 2007; 1775: 138-162

29. Ingrosso D, Cimmino A, Perna AF *et al*. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet* 2003; 361: 1693-1699

30. Perna AF, Capasso R, Lombardi C, Acanfora F, Satta E, Ingrosso D. Hyperhomocysteinemia and macromolecule modifications in uremic patients. *Clin Chem Lab Med* 2005; 43: 1032-1038

31. Stenvinkel P, Karimi M, Johansson S *et al*. Impact of inflammation on epigenetic DNA methylation - a novel risk factor for cardiovascular disease? *J Intern Med* 2007; 261: 488-499

32. Schleicher E, Friess U. Oxidative stress, AGE, and atherosclerosis. *Kidney Int Suppl* 2007; S17-S26

33. Ito A, Egashira K, Narishige T, Muramatsu K, Takeshita A. Angiotensin-converting enzyme activity is involved in the mechanism of increased endogenous nitric oxide synthase inhibitor in patients with type 2 diabetes mellitus. *Circ J* 2002; 66: 811-815

34. Chen JW, Hsu NW, Wu TC, Lin SJ, Chang MS. Long-term angiotensin-converting enzyme inhibition reduces plasma asymmetric dimethylarginine and improves endothelial nitric oxide bioavailability and coronary microvascular function in patients with syndrome X. *Am J Cardiol* 2002; 90: 974-982

35. Alkhenizan AH, Al-Omran MA. The role of vitamin E in the prevention of coronary events and stroke. Meta-analysis of randomized controlled trials. *Saudi Med J* 2004; 25: 1808-1814

36. Bleys J, Miller ER, III, Pastor-Barriuso R, Appel LJ, Guallar E. Vitamin-mineral supplementation and the progression of atherosclerosis: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2006; 84: 880-887

37. Albert CM, Cook NR, Gaziano JM *et al*. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. *JAMA* 2008; 299: 2027-2036

38. Jamison RL, Hartigan P, Kaufman JS *et al*. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007; 298: 1163-1170

39. Boaz M, Smetana S, Weinstein T *et al*. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 2000; 356: 1213-1218

40. Del RD, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 2005; 15: 316-328

41. Comporti M, Signorini C, Arezzini B, Vecchio D, Monaco B, Gardi C. F2-isoprostanes are not just markers of oxidative stress. *Free Radic Biol Med* 2008; 44: 247-256

42. Vanholder R, Massy Z, Argiles A, Spasovski G, Verbeke F, Lameire N. Chronic kidney disease as cause of cardiovascular morbidity and mortality. *Nephrol Dial Transplant* 2005; 20: 1048-1056

43. Clinical reality of coronary prevention guidelines: a comparison of EUROASPIRE I and II in nine countries. EUROASPIRE I and II Group. European Action on Secondary Prevention by Intervention to Reduce Events. *Lancet* 2001; 357: 995-1001

44. Tonelli M, Bohm C, Pandeya S, Gill J, Levin A, Kiberd BA. Cardiac risk factors and the use of cardioprotective medications in patients with chronic renal insufficiency. *Am J Kidney Dis* 2001; 37: 484-489

45. Wheeler DC, Townend JN, Landray MJ. Cardiovascular risk factors in predialysis patients: baseline data from the Chronic Renal Impairment in Birmingham (CRIB) study. *Kidney Int Suppl* 2003; S201-S203

46. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; 348: 383-393

47. Vale MJ, Jelinek MV, Best JD *et al*. Coaching patients On Achieving Cardiovascular Health (COACH): a multicenter randomized trial in patients with coronary heart disease. *Arch Intern Med* 2003; 163: 2775-2783

48. Jamison RL, Hartigan P, Kaufman JS *et al*. Effect of homocysteine lowering on mortality and vascular disease in advanced chronic kidney disease and end-stage renal disease: a randomized controlled trial. *JAMA* 2007; 298: 1163-1170

49. Kielstein JT, Zoccali C. Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age? *Am J Kidney Dis* 2005; 46: 186-202

50. Yang TL, Chen MF, Xia X, Luo BL, Li YJ. Effect of fenofibrate on the level of asymmetric dimethylarginine in individuals with hypertriglyceridemia. *Eur J Clin Pharmacol* 2006; 62: 179-184

51. Yang TL, Chen MF, Luo BL, Xie QY, Jiang JL, Li YJ. Fenofibrate decreases asymmetric dimethylarginine level in cultured endothelial cells by inhibiting NF-kappaB activity. *Naunyn Schmiedebergs Arch Pharmacol* 2005; 371: 401-407

52. Wang TD, Chen WJ, Cheng WC, Lin JW, Chen MF, Lee YT. Relation of improvement in endothelium-dependent flow-mediated vasodilation after rosiglitazone to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome. *Am J Cardiol* 2006; 98: 1057-1062

53. Scalera F, Martens-Lobenhoffer J, Bukowska A, Lendeckel U, Tager M, Bode-Boger SM. Effect of telmisartan on nitric oxide--asymmetrical dimethylarginine system: role of angiotensin II type 1 receptor gamma and peroxisome proliferator activated receptor gamma signaling during endothelial aging. *Hypertension* 2008; 51: 696-703

54. Lu TM, Ding YA, Leu HB, Yin WH, Sheu WH, Chu KM. Effect of rosuvastatin on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia. *Am J Cardiol* 2004; 94: 157-161

55. Beltowski J, Kedra A. Asymmetric dimethylarginine (ADMA) as a target for pharmacotherapy. *Pharmacol Rep* 2006; 58: 159-178

56. Axelsson J, Stenvinkel P. Role of fat mass and adipokines in chronic kidney disease. *Curr Opin Nephrol Hypertens* 2008; 17: 25-31

57. Guebre-Egziabher F, Drai J, Fouque D. Adiponectin and chronic kidney disease. *J Ren Nutr* 2007; 17: 9-12

58. Axelsson J. Obesity in chronic kidney disease: good or bad? *Blood Purif* 2008; 26: 23-29

59. Gal-Moscovici A, Sprague SM. Role of vitamin D deficiency in chronic kidney disease. *J Bone Miner Res* 2007; 22 Suppl 2: V91-V94

60. Fukagawa M, Kazama JJ. With or without the kidney: the role of FGF23 in CKD. *Nephrol Dial Transplant* 2005; 20: 1295-1298

61. Schiavi SC, Kumar R. The phosphatonin pathway: new insights in phosphate homeostasis. *Kidney Int* 2004; 65: 1-14

62. Shimada T, Hasegawa H, Yamazaki Y *et al*. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 2004; 19: 429-435

63. Zoccali C. Traditional and emerging cardiovascular and renal risk factors: an epidemiologic perspective. *Kidney Int* 2006; 70: 26-33

64. Ritz E. The clinical management of hyperphosphatemia. *J Nephrol* 2005; 18: 221-228

65. Kalantar-Zadeh K, Block G, Humphreys MH, Kopple JD. Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int* 2003; 63: 793-808